THE CHEMICAL COMPONENTS OF THE TEST OF AN AUSTRALIAN LAC INSECT AUSTROTACHARDIA ACACIAE (Maskell) (Homoptera : Lacciferidae)

by Harry F. Lower*

[Read 11 September 1958]

SUMMARY

The test of *A. acaciae* consists of chitin, protein, a dye, a wax, and a complex of lacs and lac-like substances. Of the latter which constitute more than half the dry weight of the test, none could be identified as shellac. The dye and wax are also chemically distinct from those described from other insects.

INTRODUCTION

Among the Homoptera, certain groups within the super-family, Coccoidea, are characterized by their secretion of large quantities of various waxes, or resinous materials, or both, which they incorporate into their tests or "scales". This mode of forming a protective covering for the body is most highly developed in the Lacciferidae or "lac insects" of which *Laccifer lacca* (Kerr), the Indian lac insect, is the best-known species.

There is an extensive literature dealing with its most important commercial product, shellac, and scattered references to insect waxes and dyes are to be found. The literature of the two latter has recently been reviewed by Warth (1956) and Fox (1953), respectively, while detailed accounts of the manufacture, physical and chemical constants, and industrial applications of shellac, such as those of Gardner (1937) and Parry (1925), are numerous. Apart from Fox (*op. cit.*) whose interest is in the chemistry of animal dyes generally, and Chamberlain (1923, 1925) who has provided the only complete taxonomic study of the family, the literature is entirely technological. Not only is this so, but its scope is limited to discussing three components of the test—shellac, wax and dye—and these of the one species *L. lacca*. Of the materials constituting the remainder of its test, or of any of the components of the tests of other lacciferids, nothing is known.

Austrotachardia acaciae (Maskell) is an endemic lac insect which is widely but irregularly distributed throughout the dry inland parts of Australia, where the environmental conditions admit of the growth of its host tree, *Acacia aneura* F. Muell. (mulga). The female secretes a thick, hard, brittle, dull orange-red test (Plate 1) which, in addition to the normal chitin-protein complex, contains over 60 per cent. of a variety of complex organic substances.

The material studied was collected from mulga trees on Yudnapinna Station, 50 miles N.W. of Port Augusta. Within the time at my disposal, practical difficulties made impossible the collection of material in quantity adequate for the complete examination of all substances present. Considerable distances often separate affected trees, rarely are parts of more than one or two boughs of any one tree infested, and in their colonies the insects are relatively dispersed. A further restriction of yield was imposed by the need for confining selection to

* Waite Agricultural Research Institute, University of Adelaide, South Australia.

dead insects, to obviate contamination of the sample with the body fats and fluids of living ones. Some extraneous matter was thereby unavoidably introduced in the form of desert dust, and the webbing and frass of spiders and larval scavengers. While the proportion of these was greatly reduced by subsequent treatment, it is almost certain that the greater part of the inorganic matter found was of external origin. Somewhat less than 50 g. of crude material were collected from which about 35 g. of sample were prepared.

EXPERIMENTAL

(To obviate repetition throughout the paper attention is drawn to the following:

1. All drying was done to constant weight at 103° C.
2. "Ethanol" means absolute ethanol unless otherwise specified.
3. The substantive, *laccoid*, has been coined for substances which, while exhibiting many of the properties of the lacs, show by their mode of formation that they have much in common with fatty acids.)

The crude material was dried and the resulting cake, after breaking, was ground and as much as possible passed through a sieve of mesh diameter 0·246 mm. This eliminated webbing, and wood and leaf debris. The resulting powder was vigorously stirred with water in a tall cylinder and allowed to stand until the denser fraction had settled. The floating matter was then skimmed off, dried, and re-ground. This was the sample with which all work was done. Microscopic examination of the sludge showed it to consist almost entirely of silt.

Three 10 g. portions of the sample were individually Soxhlet-extracted with ether for 30 hr. The extracts were united, the ether distilled off, and the residue collected (*Extract A*).

The residues in the thimbles were then further extracted with ethanol for 40 hr. The extracts were united and evaporated to dryness (*Extract B*).

The residues in the thimbles, after drying, were digested under reflux for 10 hr. with boiling 5 per cent. hydrochloric acid, filtered under pressure, and washed with hot water until free from acid. Filtrate and washings were evaporated to dryness (*Extract C*).

The residue was digested under reflux for 6 hr. with 200 ml. of 0·1 molar boiling sodium carbonate solution. The mixture was filtered under pressure and the residue washed with hot water until the washings were free from carbonate. Washings and filtrate were evaporated to dryness (*Extract D*).

The residue was dried and weighed. It was then ashed, and the ash weighed.

The ash was boiled in three changes of *aqua regia*, each for 15 min. After each boiling, the insoluble matter was allowed to settle and the liquid decanted. The three extracts were united and evaporated to dryness (*Extract E*).

The residue remaining after treatment with *aqua regia* was heated to redness for 5 min., cooled and weighed.

EXTRACT A (Ether-soluble)

Extract A was a soft, deep orange-brown solid. It was boiled under reflux for 12 hr. with 200 ml. of a proprietary wax solvent of high efficiency (see note at end). After cooling, the clear yellow solution was decanted and the residue boiled with three successive 50 ml. portions of the same solvent, each for 6 hr. The final extract was colourless and a drop of it evaporated without residue. The extracts were united and the solvent distilled off. The wax, after solidification, was twice recrystallized from a hot mixture of equal parts of chloroform and ethanol (charcoal).

After extraction of the wax, the residue was finely ground, well stirred with cold chloroform and filtered. The filtrate was evaporated, dissolved in ethanol, activated charcoal was added, and the mixture filtered. Evaporation of the solution gave a pure lac (Lac I).

The residue remaining after treatment with chloroform was dried, dissolved in 10 ml. of ethanol and sufficient N/10 ethanolic potassium hydroxide solution added to convert the original dye present into a potassium compound (insoluble in ethanol). The mixture was filtered and the potassium dye washed with hot ethanol until free from lac and alkali. The potassium dye was then dried, dissolved in 10 ml. of water and a slight excess of N/10 hydrochloric acid added to re-form the original dye which precipitated. The dye was extracted with ether, the solution washed twice with water, and the ether evaporated. The dye was twice recrystallized from hot chloroform.

THE WAX

The wax is soft and pale yellow in colour. Its melting point is 60·2° C. It has an acid value of 95, a saponification value of 235, an ester value of 140, and an iodine number (Hübl) of 32·3. Approximately 6 per cent. of it is unsaponifiable. This fraction consists of a hard, faintly coloured, wax-like material of melting point 70·4° C. Lack of adequate material made further investigation of the wax impracticable.

LAC I

Lac I is a dark, reddish-black, very hard lac which is brittle and breaks with a conchoidal fracture. It is very soluble in ethanol, chloroform or ether, but is insoluble in water, acetone or liquid hydrocarbons.

It has an acid value of 145, a saponification value of 302 and an ester value of 157. About 3 per cent. of it is unsaponifiable and consists of a hard, cream-coloured, wax-like solid melting at 83·1° C.

After removal of the unsaponifiable fraction, the solution was acidified and again extracted with ether. On evaporation of this extract, the lac acids remained as a soft, brownish-white, sticky mass comprising 55·6 per cent. of the weight of lac used. They were recrystallized several times from acetone (charcoal) and formed thin colourless plates having a melting point of 55·5° C.

THE DYE

The dye is apparently present as the dye acid. It is insoluble in water or acids, irrespective of temperature. Hot concentrated sulphuric acid chars it; hot concentrated nitric acid vigorously oxidizes it. It is readily soluble in ether, ethanol of any concentration higher than 60 per cent., and somewhat less so in hot chloroform. On cooling its solution in the latter, the dye separates as glittering scarlet rhombic crystals. Depending on concentration, the colour of its solutions varies from dark blood red to yellow. Its absorption spectrum in ethanolic solution is shown in Fig. 1.

On addition of sufficient ethanolic alkali to solutions of the original dye, a compound of dye and alkali precipitates. This is apparently insoluble in all liquids except water in which it is highly soluble. The colour of the solution, depending on concentration, varies from blackish violet to pale violet. Addition of ethanol to the solution precipitates the dye compound as a black, microcrystalline solid; addition of acids precipitates the original dye. From the almost black saturated aqueous solution, the potassium compound crystallizes as black, glittering prismatic needles having a violet reflexion. Its absorption spectrum in aqueous solution is shown in Fig. 2.

Over the range pH 6·9 to 8·5, its colour changes from orange, through red, to violet. The colour is red at about pH 7·8 to 7·9.

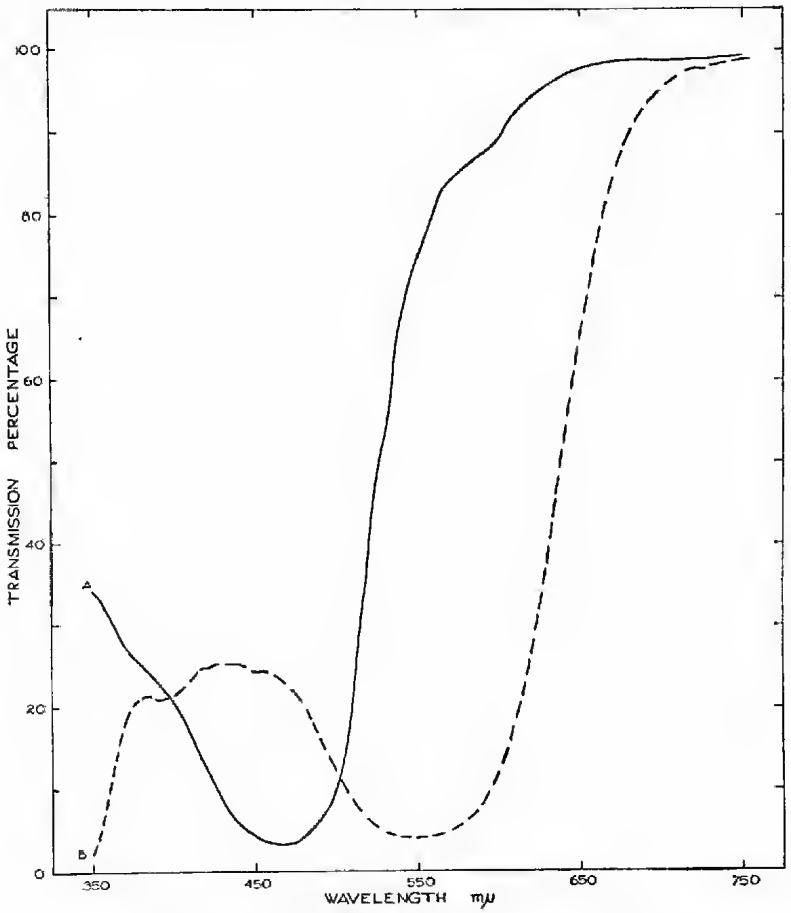

Fig. 1.—Absorption spectra of dye of *A. acaciae*.
A. Original dye in ethanolic solution. Concentration: 0·03125 g./litre.
B. Potassium compound in aqueous solution. Concentration: 0·03125 g./litre.

EXTRACT B (Ethanol-soluble)

The solid dark brown material was finely ground, digested under reflux for 10 hr. with boiling ethanol, and the residue twice digested (each for 5 hr.) with fresh boiling ethanol. The three extracts were united, activated charcoal was added, the mixture filtered and the filtrate evaporated to dryness (Lac II).

When ethanolic extraction was complete, about 54 per cent. of the original extract remained as a flocculent material closely resembling precipitated copper ferrocyanide in appearance. This was dried and weighed (Gel lac).

LAC II

Lac II is a hard, brittle, bright orange lac, very similar in appearance to orange shellac. It melts between 140° C. and 142° C. Very soluble in ethanol and somewhat less so in methanol, it is insoluble in any other of the commonly

used organic solvents. It has an acid value of 95, a saponification value of 246, and an ester value of 151. The unsaponifiable fraction comprised 2·4 per cent. and consisted of a hard, yellowish, wax-like material melting at 96·1° C.

After removal of the unsaponifiable material by ether extraction, the mixture was acidified and the resulting lac acid extracted with ether; the ether extract was evaporated and the residue thrice recrystallized from hot acetone (charcoal) forming golden-yellow, glittering scales whose melting point was 107·3° C. On cooling the melted material, it solidified as a hard orange-yellow, transparent, resin-like mass lacking the physical properties associated with fatty acids generally. It is only slightly soluble in boiling ethanol, but is very soluble in cold ether, chloroform, carbon disulphide or boiling acetone. From its solution in the latter most of it separates on cooling.

THE GEL LAC

This consisted of a brittle, black, vesicular mass which boiling ethanol restored to the original flocculent condition. It was insoluble in any of the 43 organic solvents and solvent mixtures tested.

When the solid is heated, it does not melt but decomposes into a spongy, carbonaceous mass evolving a dark dense vapour which condenses as dark red, oil-like droplets soluble in ethanol forming a reddish solution.

It was boiled with N/2 ethanolic potassium hydroxide and formed a deep, brownish-black, opaque solution which passed unchanged through filter paper. Ether extracted nothing from this solution. It was then diluted with water, placed in a separating funnel, sufficient hydrochloric acid was added to make the mixture acid, and the whole was well shaken with ether. On standing, three layers formed. The lowest consisted of an aqueous-ethanolic solution of potassium chloride coloured yellow by a trace of impurity. The middle layer was oily and black, and above this floated the orange ether layer. After running off the bottom layer, the two upper ones were well washed several times with water, allowed to stand, and then separated.

The black material was dried, dissolved in ethanol (charcoal), filtered, and again evaporated giving a black lac-like material (Laccoid I).

The ether extract was evaporated, and the residue recrystallized several times from hot acetone (charcoal) (Gel lac acid).

LACCOID I

This is a highly polished, pitch-like material, very hard and brittle, readily soluble in ethanol but insoluble in other solvents. On heating, it melts quietly at about 127° C. Boiling it with either aqueous or ethanolic alkali re-saponifies it forming a deep reddish-brown solution from which it can again be set free by acidification. Prolonged boiling with fat or wax solvents dissolves nothing from it. Its nitrogen content is 0·45 per cent.

THE GEL LAC ACID

From its solution in hot acetone, the acid separates as a deep orange, apparently amorphous, material. On heating, it softens and finally melts at about 109° C. On re-solidifying, it forms a transparent, glassy, deep orange-red, brittle solid. It dissolves readily in fat solvents and in hot acetone, but is insoluble in boiling ethanol. Aqueous or ethanolic solutions of alkalis readily re-saponify it.

EXTRACT C (Hot HCl Extract)

As first obtained, this was an orange-brown solution which, during evaporation, underwent chemical change so that the dark brown amorphous residue

could not be re-dissolved in hydrochloric acid, nor was it soluble in any other solvent tested. After boiling with ethanol, and evaporating the yellow solution, a trace of a dark brown, mucilaginous substance remained. This was dissolved in a little hot water, and the solution after decolorizing with charcoal and filtering, gave a positive result with Mölisch's reagent, but none with Fehling's solution. Since less than ·01 g. of material was available, further tests could not be performed.

The original residue contained 6·1 per cent. of nitrogen and probably consisted largely of "humin" formed by decomposition of amino-acids resulting from hydrolysis of the proteins of the test by the hydrochloric acid used for the extraction.

EXTRACT D (Sodium Carbonate Digest)

The sodium carbonate extract, on evaporation, left an almost black residue. After boiling with water, a small quantity of black insoluble matter was filtered off. Ether extracted practically nothing from the filtrate which was then acidified with hydrochloric acid. A dense precipitate formed. When the mixture was warmed, this coagulated to a yellowish-brown rubber-like mass. The mixture was then evaporated to dryness and the residue digested three times (each for 5 hr.) under reflux with boiling ether. The extracts were united and the ether distilled off, leaving a lac acid.

After expelling remaining ether, the residue was ground, dissolved in ethanol (charcoal), filtered and evaporated (Laccoid II).

THE LAC ACID

The crude lac acid was a soft, orange-coloured, wax-like material. It was several times recrystallized from hot acetone (charcoal) and finally obtained as almost colourless plates (melting point 68·2° C.). When the acid was melted and allowed to solidify it formed a soft, cream-coloured, waxy material.

LACCOID II

This was a very hard, black, lac-like material practically insoluble in all liquids except ethanol and methanol. It is extremely soluble in the former. Boiling with aqueous or ethanolic alkalis quickly brings about its saponification. From the solution it can be recovered by acidification. On heating, the solid does not melt but swells, bubbles, and evolves dense fumes which on condensing form an oil-like stain easily soluble in liquid hydrocarbons. Its nitrogen content is 2·3 per cent.

RESIDUE REMAINING AFTER SODIUM CARBONATE EXTRACTION

The residue left after sodium carbonate extraction, when dried, was a white material resembling bleached paper-pulp. It was weighed, ashed, and the ash then weighed. The loss in weight was assumed to be chitin.

The ash, after treatment with *aqua regia* (presumed to be silica) was weighed.

After weighing, the dry *aqua regia* extract was dissolved in dilute hydrochloric acid and the solution tested qualitatively for inorganic ions. The following were identified: Na^+, K^+, Ca^{++}, Mg^{++}, Fe^{+++}, PO_4^{---}, and SO_4^{--}.

DISCUSSION

The major components of the test of *A. acaciae* are shown in Table I. Since the dye, wax, and lacs I and II were separated in a relatively pure state, their proportions are reasonably correct. The "gel lac" and the acid and sodium carbonate extracts are mixtures of at least two and probably more substances. Evidence obtained during the investigation proved that had more material been

available, the diversity of substances identified would have been much greater. Frequently, traces only of certain organic compounds were isolated, the quantities of which were too small for anything other than a very general classification. The figures for silica and the inorganic ions are artificial; these materials are almost certainly of extraneous origin and form no intrinsic part of the test.

TABLE 1.

Principal constituents of the test of *A. acaciae*.

Component	Weight in grams	Percentage of weight of test
Dye	0·594	2·0
Wax	2·346	7·8
Lac I	3·935	13·1
Lac II	4·313	14·4
"Gel Lac"	5·027	16·7
"Humin"	4·050	13·5
Sodium carbonate extract	3·350	11·2
"Chitin"	4·038	13·5
Silica	0·984	3·3
Inorganic ions	0·101	0·3
Loss	1·262	4·2
	30·000	100·0

Since no corresponding study of any other lac insect has been published, little comparison with allied forms is possible. The wax and the dye are both chemically distinct from those of *Laccifer lacca* (see Warth, 1956; Fox, 1953), and the dye differs from any which has been described from other insects. Of the various members of the lac complex present, none is shellac as is shown by their solubilities, and acid, saponification, and ester values (see Gardner, 1937; Parry, 1925). The two laccoids separated are interesting compounds. In their general behaviour they resemble high melting point lacs, but their mode of chemical formation indicates a relationship to the lac acids. They are not present in the test as laccoids since their ready solubility in ethanol would result in their extraction earlier in the analysis.

As the name "lac insects" implies, production of lacs is characteristic of the Lacciferidae. In *A. acaciae* they comprise over half the dry weight of the test, but their biological significance in any species has never been explained, little is known of their mode of secretion, and nothing of their metabolism or function.

NOTE.—The proprietary wax solvent mentioned above is marketed by the Vacuum Oil Company as "Stanvac Hexane". It consists of 93-95 per cent. saturated hydrocarbons and 7-5 per cent. of aromatic hydrocarbons. The boiling point range (A.S.T.M.) is from 66° C. to 68·5° C.

REFERENCES

CHAMBERLIN, J. C., 1923. A systematic monograph of the Tachardiinae or lac insects (Coccidae). Bull. ent. Res., 14, pp. 147-212.

CHAMBERLIN, J. C., 1925. Supplement to a monograph of the Lacciferidae (Tachardiinae) or lac insects (Homopt., Coccidae). Bull. ent. Res., 16, pp. 31-41.

FOX, D. L., 1953. Animal Biochromes and Structural Colours. Cambridge University Press, pp. 205-208.

GARDNER, H. A., 1937. Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors. Eighth edition. Institute of Paint and Varnish Research, Washington, D.C., pp. 873-902.

PARRY, E. J., 1925. In Allen's Commercial Organic Analysis. Fifth edition. Churchill, London, pp. 290-299.

WARTH, A. H., 1956. The Chemistry and Technology of Waxes. Second edition. Reinhold Publishing Corp., New York, pp. 76-121.